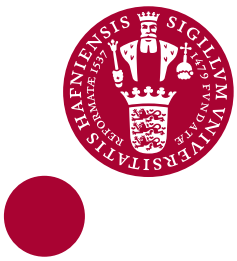

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ORIGINAL RESEARCH

Insulin-Like Growth Factor Binding Protein 4 Fragments Provide Incremental Prognostic Information on Cardiovascular Events in Patients With ST-Segment Elevation Myocardial Infarction

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***Background*—**Fragments of insulin-like growth factor binding protein 4 (IGFBP-4) are potential new biomarkers for cardiac risk assessment. The fragments are generated on specific cleavage by pregnancy-associated plasma protein-A, which exerts proatherogenic activity. This study investigated the prognostic value of IGFBP-4 fragments in patients with ST-segment elevation myocardial infarction.

***Methods and Results*—**We prospectively included 656 patients with ST-segment elevation myocardial infarction treated with percutaneous coronary intervention from September 2006 to December 2008. Blood samples were drawn before percutaneous coronary intervention, and levels of intact IGFBP-4 and N-terminal and C-terminal IGFBP-4 fragments were measured by specific assays. End points were 5-year all-cause and cardiovascular mortality and the combined end point of major adverse cardiac events. Prognostic potential was evaluated on top of a clinical model in terms of discrimination, calibration, and reclassification analysis. During follow-up, 166 patients experienced a major adverse cardiac event and 136 patients died, of whom 69 died from cardiovascular causes. Both IGFBP-4 fragments were associated with all end points ($P<0.001$). After multivariable adjustments, both N-terminal and C-terminal IGFBP-4 fragment levels remained associated with all end points, including cardiovascular mortality with hazard ratios per doubling in protein concentration of 2.54 (95% CI 1.59–4.07; $P<0.001$) and 2.07 (95% CI 1.41–3.04; $P<0.001$), respectively. Incorporation of IGFBP-4 fragments into a clinical model with 15 risk factors improved C-statistics and model calibration and provided incremental prognostic contribution, as assessed by net reclassification improvement and integrated discrimination improvement.

***Conclusions*—**IGFBP-4 fragments are associated with increased risk of all-cause mortality, cardiovascular mortality, and major adverse cardiac events in patients with ST-segment elevation myocardial infarction. **(*J Am Heart Assoc*. 2017;6:e005358. DOI: 10.1161/JAHA.116.005358.)**

Key Words: biomarker • cardiovascular disease • insulin-like growth factor binding protein 4 • pregnancy-associated plasma protein A • ST-segment elevation myocardial infarction

An important objective of cardiovascular disease (CVD) research is to identify novel biomarkers to improve stratification of patients with excessive risk. Pregnancy-associated plasma protein-A (PAPP-A) has been regarded a candidate marker in CVD.[1,2] PAPP-A is a metzincin metalloproteinase present in the circulation as well as within tissues, where it reversibly adheres to cell surfaces.[3] In 2001, PAPP-A was shown to be ubiquitously present in eroded atherosclerotic plaques, and circulating levels were elevated in patients with acute coronary syndrome.[1] It was later revealed, however, that administration of unfractionated heparin to patients results in an abrupt increase in PAPP-A

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Accompanying Table S1 and Figure S1 are available at http://jaha.ahajournals.org/content/6/3/e005358/DC1/embed/inline-supplementary-material-1.pdf

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concentrations, probably through displacement of cell surface–tethered PAPP-A.[4,5] Because heparin is part of the standard initial treatment of patients with acute myocardial infarction, samples from these patients are not suitable for PAPP-A measurements.

The proteolytic activity of PAPP-A is directed primarily toward insulin-like growth factor (IGF) binding protein 4 (IGFBP-4), which is a key regulator of IGF bioactivity.[6–8] On local PAPP-A–mediated cleavage of IGFBP-4, IGF accessibility is increased in proximity to the IGF-1 receptor. IGF-1 stimulates cell proliferation and promotes macrophage activation, low-density lipoprotein uptake, and release of proinflammatory cytokines, which likely facilitate atherogenesis and plaque instability.[9] The functional role of PAPP-A in the process of plaque destabilization has been confirmed in PAPP-A transgenic and gene knockout mouse models,[10,11] and these findings further support a proatherogenic effect of PAPP-A–mediated IGF-1 release.

It has been proposed that the PAPP-A–generated IGFBP-4 fragments may be reflective of PAPP-A enzymatic activity. Circulating levels of total PAPP-A correlate with levels of N-terminal IGFBP-4 (NT-IGFBP-4) and C-terminal IGFBP-4 (CT-IGFBP-4)[12]; therefore, IGFBP-4 fragments released from the atheromatous plaque could reflect plaque burden and thus serve as markers of atherosclerosis.[13] We recently verified that circulating IGFBP-4 fragment levels are unaffected by heparin treatment of patients with ST-segment elevation myocardial infarction (STEMI),[5] and no other medical therapies are known to significantly impinge IGFBP-4 fragment levels.

The aim of the present study was to examine the association between PAPP-A–generated IGFBP-4 fragments and risk of mortality and major adverse cardiac events (MACE) in a cohort of patients with STEMI. The value of the IGFBP-4 fragments was compared with traditional cardiovascular risk factors in terms of discrimination, calibration, and reclassification analysis.

Materials and Methods

Patients

A total of 656 patients with STEMI treated with primary percutaneous coronary intervention at Gentofte University Hospital, Denmark, were included from September 2006 through December 2008. Details of the study population have been described previously.[14] Inclusion criteria were onset of STEMI symptoms, including chest pain lasting for >30 minutes and <12 hours, persistent ST-segment elevation of ≥2 mm in ≥2 contiguous precordial ECG leads or ≥1 mm in ≥2 contiguous limb ECG leads, and an increase in troponin I (TnI) to >0.5 μg/L. All patients were pretreated with unfractionated heparin, and glycoprotein IIb/IIIa inhibitors were administered at the discretion of the operator. Following the percutaneous coronary intervention procedure, patients received aspirin (75 mg/day), clopidogrel (75 mg/day for 12 months), cholesterol-lowering treatment (statins), and β-receptor antagonists. Hypertension and hypercholesterolemia were defined as use of blood pressure–lowering or cholesterol-lowering drugs, respectively, on admission. Patients were considered to have diabetes mellitus if they received glucose-lowering drugs on admission or had a fasting plasma glucose level ≥7 mmol/L or a nonfasting level of ≥11.1 mmol/L. Multivessel disease was defined as 2- or 3-vessel disease and complex lesions such as type C lesions. Advanced echocardiography was performed in 344 patients (52%) at 1 to 3 days after admission using Vivid 7 or E9 (GE Healthcare). Left ventricular ejection fraction (LVEF) was obtained using the modified biplane Simpson method. Serum and EDTA plasma were drawn from the femoral sheath at the beginning of the percutaneous coronary intervention procedure and stored at −80°C. The study was approved by the local ethics committee and the Data Protection Agency and complied with the Declaration of Helsinki. Informed consent was obtained from all participants.

Follow-up and End Points

Patients were prospectively followed for 5 years through the Danish Civil Registration system and the National Causes of Death Registry, which offers information from physicians on causes of death according to the *International Classification of Diseases, 10th Revision* (ICD-10).[15] Follow-up information was collected on all-cause mortality, death due to recorded cardiovascular event, and occurrence of nonfatal MACE defined as readmission due to acute myocardial infarction, ischemic stroke, or heart failure. Information on readmissions was obtained from the highly validated National Patient Registry using the ICD-10 codes.[16] Information was obtained for all patients, and all events were thoroughly validated using medical records, including laboratory measurements, hospital summaries, and operative reports.

Laboratory Measurements

Routine methods

High-sensitivity C-reactive protein was determined by a nephelometric assay (Dade Behring), and creatinine was assayed using standard laboratory methods. TnI levels were determined at baseline and after 6 and 12 hours using the Immulite 2500 STAT Troponin I immunoassay (Siemens Healthcare). Later determinations were performed if deemed necessary, and the highest obtained value was reported as peak TnI. Estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease formula.[17]

Intact IGFBP-4 and NT- and CT-IGFBP-4 fragments

EDTA plasma levels of IGFBP-4, CT-IGFBP-4, and NT-IGFBP-4 were measured in duplicate using in-house time-resolved immunofluorometric assays based on monoclonal antibodies (mAb) and recombinant human (rh) calibrators generously provided by HyTest Ltd. The assays were performed as recently described.[5] Intact IGFBP-4 was measured using coating mAb IBP182 (4IGF4 IBP182) and detection mAb IBP144 (4IGF4EU IBP144). mAb IBP3 (4IGF4 IBP3) and mAb IBP180 (4IGF4EU IBP180) were used for the determination of the NT-IGFBP-4 fragment, and mAb IBP182 and mAb IBP163 (4IGF4EU IBP163) were applied for the determination of the CT-IGFBP-4 fragment. As calibrators, full-length rhIGFBP-4 (8IGF4), rhNT-IGFBP-4 (8NFB4), and rhCT-IGFBP-4 (8CIG4) were applied. In each fragment assay, one of the antibodies specifically recognized the proteolytic neoepitope generated on cleavage by PAPP-A. Detection limits were 0.5 μg/L IGFBP-4, 0.4 μg/L CT-IGFBP-4, and 0.9 μg/L NT-IGFBP-4. Intra- and interassay coefficient of variations (CVs) were <10% and <15%, respectively.

Statistical Analysis

Nonnormally distributed variables were $\log_2$-transformed prior to statistical analyses. Frequency distribution of $\log_2$-NT- and $\log_2$-CT-IGFBP-4 and fitted normal curves are provided in Figure S1. A combined end point including all events was used for comparison of baseline characteristics. Patients with or without events during follow-up were compared using the Student *t* test or Mann–Whitney *U* statistics on continuous variables, and the chi-square test on categorical variables. The Bonferroni adjusted level of significance was reported for correlation coefficients (*r* value). Receiver operating characteristic (ROC) curves were used to analyze the prognostic values of intact IGFBP-4, NT-IGFBP-4, CT-IGFBP-4, C-reactive protein, and peak TnI. C-statistics between models were compared by testing equality concordance using Mann–Whitney *U* statistics.[18] Kaplan–Meier survival curves were performed for NT- and CT-IGFBP-4 quartiles, and incidence distributions were compared using the log-rank test.

Model Building

Survival analyses were performed using Cox proportional hazards models. All candidate confounder variables and blood biomarkers (Table 1) were initially considered for the model, as were all transformations and interactions between them. In model 1, prespecified variables were included based on traditional cardiovascular risk factors (age, sex, systolic blood pressure, total cholesterol level, high-density lipoprotein level, diabetes mellitus, and smoking status).[19] Furthermore, a criterion for inclusion in model 1 was minimization of the Bayesian information criterion.[20] The Bayesian information criterion is a likelihood-based measure for which lower values indicate better model fit and in which a penalty is paid for an increased number of variables. Finally, a covariate was included if it changed the estimate measure of association by >10%. A baseline clinical model was generated including all variables selected for model 1 (age, sex, systolic blood pressure, total cholesterol level, high-density lipoprotein, diabetes mellitus, smoking status, estimated glomerular filtration rate, hypertensive treatment, hemoglobin, previous acute myocardial infarction, multivessel coronary artery disease, complex lesion, C-reactive protein, and peak TnI). The clinical model was then extended by the $\log_2$-transformed NT- or CT-IGFBP-4 variable, and results were reported as hazard ratio (HR) and 95% CI. Accordingly, each unit increase in NT- or CT-IGFBP-4 on the $\log_2$-scale corresponds to a doubling in NT- or CT-IGFBP-4, respectively. Because LVEF measurements were obtained in only 344 patients, model 1 was assessed with and without LVEF as a covariate. Body mass index, hypercholesterolemia, low-density lipoprotein, triglyceride, symptom-to-balloon time, and use of glycoprotein IIb/IIIa inhibitor did not fulfill the criteria for inclusion but were included in a second model (model 2) as an extension of model 1. Hazard proportionality and linearity assumptions were checked by log-log plots, fitted survival curves, and smoothed martingale and Schonenfeld residuals plots.[21–23] Furthermore, because a large number of events occurred within the first 3 months after STEMI, survival analyses were repeated using a 3-month end point.

Model Performances

The incremental value of adding $\log_2$-NT- or $\log_2$-CT-IGFBP-4 to the clinical model for predicting end points was evaluated. Discriminatory abilities of the models were compared by Harrell's *C* and Somers' *D* statistics for censored data using the "somersd" module in Stata version 13 (StataCorp LP). For calibration, the Royston modification of Nagelkerke's R^2 statistic for proportional hazards models was used to assess the explained variation by using 1000 bootstrap repetitions of the whole data set through the "str2ph" module in Stata.[24] The Groennesby and Borgan extension of the Hosmer–Lemeshow goodness-of-fit test was applied to assess how well the predicted models fitted the observed incidence data.[25] Well-calibrated models show no significant differences in the Hosmer–Lemeshow test results. The Akaike information criterion and the Bayesian information criterion were calculated for each model. Lower values indicate a better model, but no statistical test compares the Akaike information criterion and Bayesian information criterion scores. Overall goodness-of-fit was evaluated by likelihood ratio test. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI), as described by Pencina et al,[26,27]

Table 1. Baseline Characteristics

Characteristics	Total (n=656)	No Events (n=431)	Events (n=225)	*P* Value
Age, y	63±12	60±11	69±12	<0.001
Male/female, n	484/172	324/107	160/65	0.261
BMI, kg/m^2	26 (24; 29)	26 (24; 29)	26 (23; 29)	0.034
Diabetes mellitus, %	9.1	7.7	12.0	0.067
Current smoker, %	52.7	54.8	48.9	0.153
Hypercholesterolemia, %	19.4	17.2	23.6	0.049
Hypertension, %	33.8	28.8	43.6	<0.001
Blood glucose, mmol/L	8.3 (7.0; 9.9)	8.1 (7.0; 9.4)	8.7 (7.2; 11)	<0.001
Hemoglobin, mmol/L	8.7±1.0	8.8±0.8	8.5±1.1	<0.001
Total cholesterol, mmol/L	4.8±1.1	4.9±1.1	4.6±1.2	<0.001
HDL cholesterol, mmol/L	1.3±0.4	1.3±0.3	1.3±0.4	0.111
LDL cholesterol, mmol/L	2.9±1.0	3.0±1.0	2.7±1.1	<0.001
Triglycerides, mmol/L	0.98 (0.69; 1.6)	0.98 (0.67; 1.6)	0.99 (0.73; 1.5)	0.926
Systolic blood pressure, mm Hg	133±27	135±26	128±28	0.003
eGFR, mL/min/1.73 m^2	73±24	78±21	63±27	<0.001
Creatinine, μmol/L	91 (78; 109)	88 (76; 103)	101 (84; 133)	<0.001
C-reactive protein, mg/L	3 (1; 9)	3 (1; 7)	5 (2; 16)	<0.001
Peak troponin I, μg/L	90 (28; 244)	81.3 (28; 212)	117 (27; 281)	0.059
Left ventricular ejection fraction, %*	45.8±9.0	46.9±8.6	42.8±9.3	<0.001
Glycoprotein IIb/IIIa inhibitor, %	25.9	25.8	26.2	0.897
Symptom-to-balloon time, minute	195 (130; 323)	190 (123; 310)	230 (140; 355)	0.051
Multivessel disease, %	27.0	24.6	31.6	0.057
Culprit lesion, %				0.353
Left anterior descending artery	47.6	47.1	48.4	
Circumflex artery	10.4	11.6	8.0	
Right coronary artery	42.0	41.3	43.6	
Complex lesion, %	50.9	48.3	56.0	0.060
Previous myocardial infarction, %	6.3	5.1	8.4	0.093
Died during follow-up, n	136	0	136	
Died due to cardiovascular event, n	69	0	69	
MACE during follow-up, n	166	0	166	
IGFBP-4, μg/L	153±70	146±64	164±79	0.002
NT-IGFBP-4, μg/L	132 (99; 185)	120 (94; 155)	182 (118; 264)	<0.001
CT-IGFBP-4, μg/L	52 (34; 77)	45 (31; 66)	69 (46; 108)	<0.001

Data are mean±SD or median (25th percentile; 75th percentile). Categorical variables are indicated as numbers (n) or percentage (%) of patients. BMI indicates body mass index; CT, C-terminal; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IGFBP-4, insulin-like growth factor binding protein 4; LDL, low-density lipoprotein; MACE, major adverse cardiac event; NT, N-terminal.

*Left ventricular ejection fraction was available in only 344 patients (52%), of which 102 patients experienced an event.

were used to evaluate improvements in risk predictions. NRI compares the nested addition of a new variable to a reference model, rewarding correct reclassification and penalizing incorrect reclassification. Any upward movement in patients experiencing an event implies improved classification, whereas a downward movement implies worse reclassification. The interpretation is opposite for patients who do not experience an event. NRI was computed both as category-based NRI and continuous NRI. Category-based NRI was assessed by comparing the predicted 5-year risk of all-cause

mortality, cardiovascular mortality, and MACE between models across risk categories of <5%, 5% to 10%, 10% to 20%, and >20% risk. Because risk categories in STEMI have not been defined, thresholds were based on values suggested in previous studies of CVD events.[28,29] Conversely, the continuous NRI counts the direction of change in risk for every participant rather than the crossing of a threshold. The IDI is independent of category thresholds and reflects the actual extent of change and not merely the direction. NRI and IDI were obtained using 1000 bootstrap repetitions through the "incrisk" module in Stata.

Results are presented as mean±SD for normally distributed data and median (25th percentile; 75th percentile) for skewed data. C-statistics and HRs are presented with 95% CIs. A 2-tailed $P<0.05$ was considered statistically significant. Data were analyzed using Stata software.

Results

Baseline data are presented in Table 1. Median follow-up of patients was 5 years (range 9 days to 5 years), during which 225 patients (34.3%) suffered from all-cause mortality, cardiovascular mortality, or MACE. A total of 136 patients (20.7%) died, and among causes of deaths, cardiovascular events were responsible in 69 patients (10.5%). In a large number of patients, all-cause mortality (n=46), cardiovascular mortality (n=41), or MACE (n=59) occurred within the first 3 months after STEMI. Because of the high-quality Danish registration system, no patients were lost to follow-up.

Patients with events had higher circulating levels of intact IGFBP-4 as well as NT- and CT-IGFBP-4, and a strong positive correlation was observed between concentrations of NT-IGFBP-4 and CT-IGFBP-4 ($r=0.83$, $P<0.001$). IGFBP-4 fragment levels correlated negatively with estimated glomerular filtration rate (NT-IGFBP-4: $r=-0.56$, $P<0.001$; CT-IGFBP-4: $r=-0.50$, $P<0.001$); therefore, increased fragment concentrations were associated with reduced kidney function.[6,30] IGFBP-4 fragment levels were not associated with sex, diabetes mellitus, symptom-to-balloon time, culprit lesion location or complexity, peak TnI level, hypercholesterolemia, or use of glycoprotein IIb/IIIa inhibitors. LVEF did not significantly correlate with NT-IGFBP-4 ($r=-0.119$, $P=0.179$) or CT-IGFBP-4 ($r=-0.114$, $P=0.219$).

C-statistics for NT- and CT-IGFBP-4 were significantly higher compared with peak TnI levels ($P<0.001$) and C-reactive protein ($P<0.05$) for all end points (Figure 1 and Table 2). Intact IGFBP-4 did not perform better than C-reactive protein or peak TnI for any end point (data not shown), and thus was not subjected to further analyses. Kaplan–Meier survival curves for all-cause mortality, cardiovascular mortality, and MACE in patients stratified by NT- and CT-IGFBP-4 quartiles are illustrated in Figure 2. The log-rank analyses showed significantly different incidence distributions according to NT- and CT-IGFBP-4 quartiles (all $P<0.001$) (Table 2).

Cox Regression and Survival

NT- and CT-IGFBP-4 were predictors of MACE and all-cause and cardiovascular mortality as continuous variables (log_2-NT-IGFBP-4 and log_2-CT-IGFBP-4) in both univariable and multivariable analyses (Table 3). After multivariable adjustments, NT- and CT-IGFBP-4 fragment levels were associated with cardiovascular mortality with HRs per doubling in protein concentration of 2.54 (95% CI 1.59–4.07; $P<0.001$) and 2.07 (95% CI 1.41–3.04; $P<0.001$), respectively. The prognostic

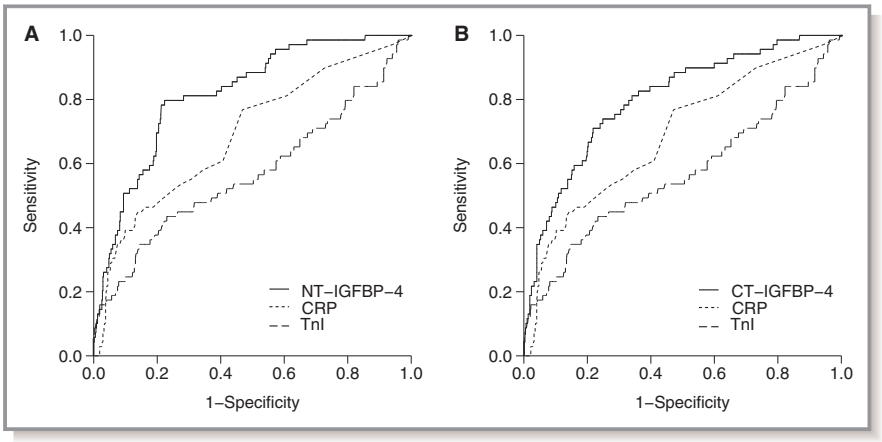

Figure 1. Receiver operating characteristic curves for CRP, peak TnI, NT-IGFBP-4 (A), and CT-IGFBP-4 (B). The end point was cardiovascular mortality. CRP indicates C-reactive protein; CT, C-terminal; IGFBP-4, insulin-like growth factor binding protein 4; NT, N-terminal; ROC, receiver operating characteristic; TnI, troponin I.

Table 2. C-Statistics and Log-Rank Analyses of All-Cause Mortality, Cardiovascular Mortality, and MACE

C-Statistics	NT-IGFBP-4	CT-IGFBP-4	CRP	Peak TnI
All-cause mortality	0.76 (0.72; 0.81)*,†	0.75 (0.70; 0.80)*,†	0.68 (0.63; 0.73)	0.55 (0.50; 0.61)
Cardiovascular mortality	0.82 (0.77; 0.87)*,†	0.80 (0.75; 0.86)*,†	0.69 (0.62; 0.76)	0.57 (0.48; 0.65)
MACE	0.71 (0.67; 0.76)*,†	0.70 (0.66; 0.75)*,†	0.59 (0.54; 0.64)	0.54 (0.49; 0.60)

	NT-IGFBP-4 Quartiles				
Log-Rank	1 (31.6–100.8 μg/L)	2 (100.9–132.6 μg/L)	3 (132.7–184.8 μg/L)	4 (184.9–810.1 μg/L)	*P* Value
Patients, n	164	164	164	164	
All-cause mortality, n	12	17	31	76	<0.001
Cardiovascular mortality, n	1	8	12	48	<0.001
MACE, n	16	32	35	83	<0.001

	CT-IGFBP-4 Quartiles				
	1 (4.1–34.3 μg/L)	2 (34.4–52.0 μg/L)	3 (52.1–78.3 μg/L)	4 (78.4–348.8 μg/L)	*P* Value
Patients, n	164	164	164	164	
All-cause mortality, n	12	16	36	72	<0.001
Cardiovascular mortality, n	4	6	14	45	<0.001
MACE, n	19	30	44	73	<0.001

C-statistics are reported individually for NT-IGFBP-4, CT-IGFBP-4, peak TnI, and CRP (mean and 95% CI). CRP, C-reactive protein; CT, C-terminal; IGFBP-4, insulin-like growth factor binding protein 4; MACE, major adverse cardiac event; NT, N-terminal; TnI, Troponin I.
*P<0.001 when compared with peak TnI.
†P<0.05 when compared with CRP. Log-rank values are reported a numbers (n) of patients.

performances were not affected by inclusion of the covariates (model 2) that did not fulfill the criteria for inclusion in model 1. In the subgroup of patients with LVEF measurements, addition of LVEF to model 1 did not alter the prognostic performance, and NT- and CT-IGFBP-4 remained significantly associated with all end points (P<0.001).

A large number of events occurred within the first 3 months after the STEMI. To investigate the association between NT- and CT-IGFBP-4 and early events, survival analyses were also performed using 3 months as the end point. In both univariable and multivariable analyses, NT- and CT-IGFBP-4 were predictors of MACE and all-cause and cardiovascular mortality at 3 months. After multivariable adjustments (model 1), cardiovascular mortality HRs per doubling in protein concentration of NT- or CT-IGFBP-4 were 1.97 (95% CI 1.48–2.61; P<0.001) and 1.70 (95% CI 1.35–2.15; P<0.001), respectively. Adjusting for the variables in model 2 resulted in HRs of 2.71 (95% CI 1.42–5.17; P<0.001) for NT-IGFBP-4 and 2.07 (95% CI 1.23–3.48; P<0.001) for CT-IGFBP-4.

Incremental Prognostic Value of IGFBP-4 Fragments Over Clinical Risk Factors

Table 4 shows the results of analysis using calibration, discrimination, and reclassification metrics to evaluate the incremental usefulness of IGFBP-4 fragments over clinical risk factors for the prediction of all-cause mortality, cardiovascular mortality, and MACE. C-statistics for the prediction of all end points significantly increased when NT- or CT-IGFBP-4 was added to the clinical model. For the prediction of cardiovascular mortality, NT- and CT-IGFBP-4 provided significant discriminatory information with increases in C-statistics of 2.9% (0.38; 5.5%; P=0.025) and 2.7% (0.15; 5.3%; P=0.038), respectively.

Nagelkerke's R^2 was higher for models extended by NT- or CT-IGFBP-4. For nested models, R^2 increases with the strength of association, and thus higher R^2 indicates better calibration of a model. The P values for the Hosmer–Lemeshow statistics signified that model-based estimates aligned with observed outcomes. Akaike information criterion and Bayesian information criterion were lower for all end points in models containing NT- or CT-IGFBP-4. Global goodness-of-fit was better in models including NT- or CT-IGFBP-4 than in the model with only established risk factors, as evaluated by likelihood ratio tests.

Category NRI, continuous NRI, and IDI were calculated to evaluate whether the addition of the IGFBP-4 fragments to the clinical model led to any significant risk reclassification of the end points. Continuous NRI and IDI were significantly increased for all end points after inclusion of either NT- or CT-IGFBP-4. Consequently, participants were correctly

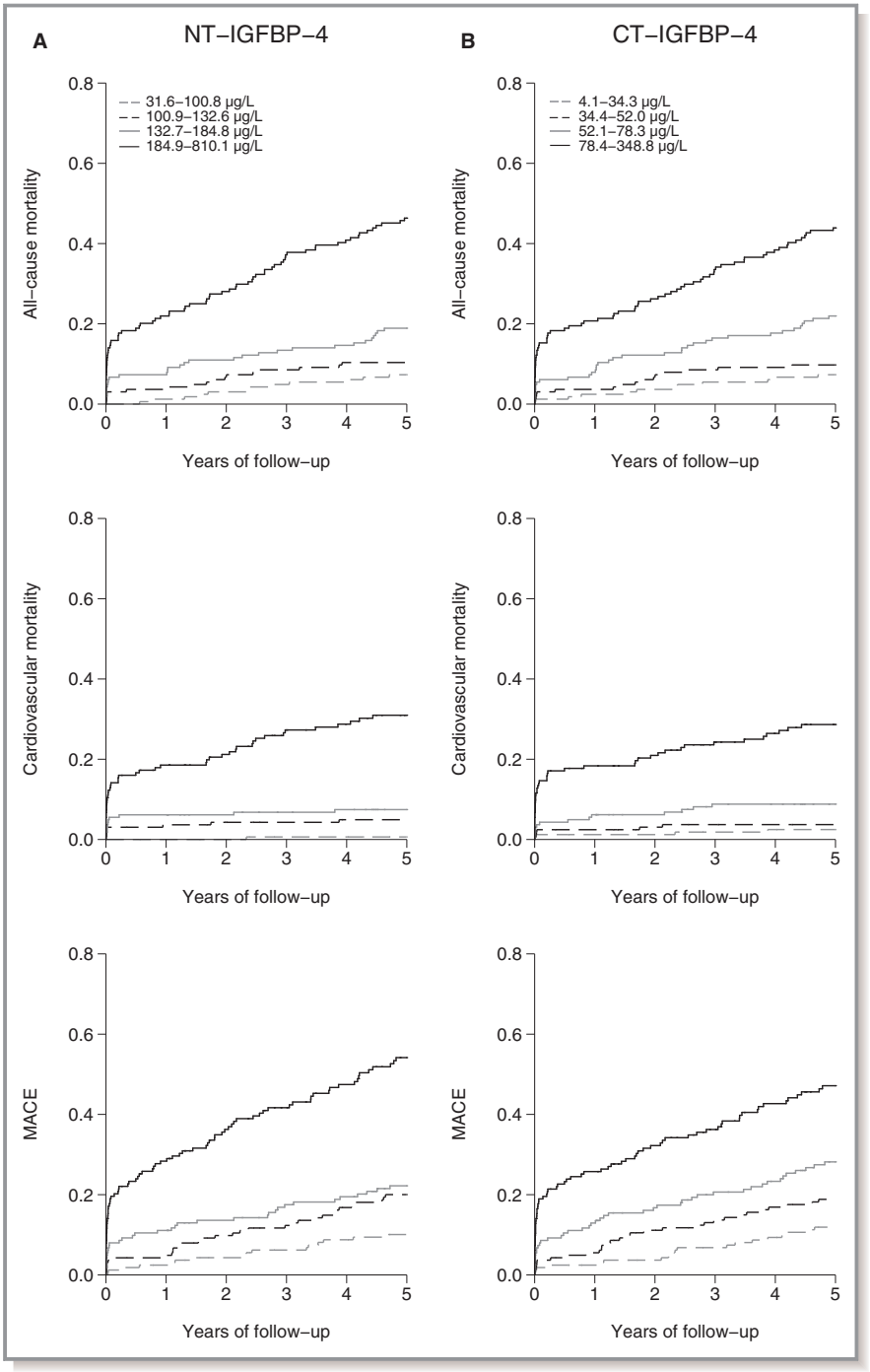

Figure 2. Risk of all-cause mortality, cardiovascular mortality, and MACE in patients according to quartiles of NT-IGFBP-4 (A) and CT-IGFBP-4 (B). CT indicates C-terminal; IGFBP-4, insulin-like growth factor binding protein 4; MACE, major adverse cardiac event; NT, N-terminal.

reclassified both according to direction of change in risk and actual extent of change. Category-based NRI using predefined risk categories of <5%, 5% to 10%, 10% to 20%, and >20% risk was significant for all end points after addition of NT-IGFBP-4 to the clinical model (95% CI did not contain zero). Of the 69 patients who died of CVD, 33.3% changed risk category when NT-IGFBP-4 was included. Of these, 78.3% were correctly reclassified to a higher risk category. Of the 587 patients not suffering from cardiovascular mortality, 27.6% changed risk category and 63.9% were correctly reclassified. The reclassification table of patients initially classified as having a 5-year risk of cardiovascular mortality based on model 1 who were

Table 3. Multivariable Cox Regression Analyses for All-Cause Mortality, Cardiovascular Mortality, and MACE at 5 Years

	All-Cause Mortality		Cardiovascular Mortality		MACE	
Model	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Log_2-NT-IGFBP-4						
All patients (n=656)						
Univariable	3.33 (2.69–4.13)	<0.001	4.36 (3.25–5.86)	<0.001	2.71 (2.22–3.30)	<0.001
Model 1	2.13 (1.53–2.97)	<0.001	2.54 (1.59–4.07)	<0.001	1.97 (1.48–2.61)	<0.001
Model 2	2.19 (1.55–3.10)	<0.001	2.70 (1.65–4.41)	<0.001	1.93 (1.45–2.58)	<0.001
Subgroup with LVEF (n=344)						
Univariable	2.17 (1.47–3.21)	<0.001	4.09 (2.08–8.03)	<0.001	2.35 (1.74–3.18)	<0.001
Model 1	1.75 (1.05–3.10)	0.031	2.62 (0.97–7.05)	0.057	1.66 (1.09–2.51)	0.017
Univariable plus LVEF	2.12 (1.42–3.15)	<0.001	3.85 (1.93–7.67)	<0.001	2.13 (1.57–2.89)	<0.001
Model 1 plus LVEF	1.72 (1.02–3.09)	0.042	2.63 (1.01–7.05)	0.047	1.59 (1.03–2.45)	0.035
Log_2-CT-IGFBP-4						
All patients (n=656)						
Univariable	2.78 (2.27–3.40)	<0.001	3.69 (2.78–4.89)	<0.001	2.38 (1.98–2.86)	<0.001
Model 1	1.74 (1.33–2.29)	<0.001	2.07 (1.41–3.04)	<0.001	1.70 (1.35–2.15)	<0.001
Model 2	1.78 (1.34–2.37)	<0.001	2.13 (1.43–3.16)	<0.001	1.70 (1.34–2.16)	<0.001
Subgroup with LVEF (n=344)						
Univariable	2.11 (1.49–2.97)	<0.001	4.29 (2.23–8.25)	<0.001	2.29 (1.73–3.03)	<0.001
Model 1	1.79 (1.11–2.89)	0.016	3.63 (1.32–9.99)	0.012	1.65 (1.16–2.35)	0.006
Univariable plus LVEF	2.06 (1.46–2.92)	<0.001	4.02 (2.07–7.82)	<0.001	2.12 (1.60–2.79)	<0.001
Model 1 plus LVEF	1.78 (1.10–2.87)	0.019	3.62 (1.31–9.95)	0.013	1.60 (1.11–2.29)	0.012

Model 1: age, sex, estimated glomerular filtration rate, hypertension, high-density lipoprotein, total cholesterol, systolic blood pressure, hemoglobin, current smoking, diabetes, previous acute myocardial infarction, multivessel coronary artery disease, complex lesion, C-reactive protein, and peak troponin I. Model 2: model 1 plus body mass index, hypercholesterolemia, low-density lipoprotein, triglyceride, symptom-to-balloon time, and use of glycoprotein IIb/IIIa inhibitor. Separate analyses were performed on the subgroup of patients with LVEF measurements (n=344). Results are reported as HR and 95% CI. One-unit increase in NT- or CT-IGFBP-4 on the log_2-scale corresponds to a doubling in NT- or CT-IGFBP-4, respectively. CT indicates C-terminal; HR, hazard ratio; IGFBP-4, insulin-like growth factor binding protein 4; LVEF, Left ventricular ejection fraction; MACE, major adverse cardiac event; NT, N-terminal.

reclassified to a higher or lower risk category by the addition of NT-IGFBP-4 is illustrated in Table S1. CT-IGFBP-4 significantly improved risk reclassification for the prediction of cardiovascular mortality but did not yield any improvements for the prediction of all-cause mortality or MACE (95% CI contained zero).

Discussion

The present study demonstrates that IGFBP-4 fragments are associated with an increased risk of all-cause mortality, cardiovascular mortality, and MACE in a cohort of patients with STEMI. The associations were significant both at 3 months and 5 years after the STEMI. Nevertheless, an association alone is insufficient to establish prognostic value, and the use of IGFBP-4 fragments to determine the risk of a future event as a continuous variable demands cautious consideration. Various risk scores and statistical tests in the cardiovascular area have generated considerable controversy, and no clear consensus has been established. In this study, the incremental prognostic value of the addition of the IGFBP-4 fragments to the clinical model was assessed by discrimination, calibration, and reclassification analyses. Discrimination and calibration analyses indicated that models including either NT- or CT-IGFBP-4 were more accurate than the clinical model alone. To assess the magnitude of improvement rather than testing the hypothesis that said improvement was greater than zero, we computed category-based NRI, continuous NRI, and IDI. Importantly, neither NT- nor CT-IGFBP-4 had negative overall NRI values, which are indicative of worsened reclassification. When assessing the true discriminatory potential of a new biomarker in contrast to other risk factors, and especially when making comparisons between studies, continuous NRI may be the best metric. It yields the incremental strength of the new biomarker after accounting for correlations with variables included in the clinical model and, unlike HRs, can be compared between studies of biomarkers with different statistical distributions. Although

Table 4. Performance of Models for All-Cause Mortality, Cardiovascular Mortality, and MACE at 5 Years

Performance Measures	All-Cause Mortality			Cardiovascular Mortality			MACE		
	Clinical Model	Clinical Model Plus log_2-NT-IGFBP-4	Clinical Model Plus log_2-CT-IGFBP-4	Clinical Model	Clinical Model Plus log_2-NT-IGFBP-4	Clinical Model Plus log_2-CT-IGFBP-4	Clinical Model	Clinical Model Plus log_2-NT-IGFBP-4	Clinical Model Plus log_2-CT-IGFBP-4
Discrimination									
C-statistic (95% CI)	0.761 (0.720–0.801) Reference	0.780 (0.742–0.818) P=0.026	0.778 (0.740–0.816) P=0.043	0.813 (0.765–0.861) Reference	0.843 (0.799–0.887) P=0.025	0.841 (0.794–0.887) P=0.038	0.704 (0.664–0.745) Reference	0.728 (0.688–0.767) P=0.018	0.726 (0.687–0.765) P=0.028
Calibration									
Overall performance									
Nagelkerke's R^2	0.470	0.526	0.517	0.667	0.723	0.721	0.287	0.350	0.347
Goodness of fit									
H-L test (χ^2)	10.86 P=0.301	5.35 P=0.803	4.29 P=0.886	4.53 P=0.874	3.46 P=0.943	13.2 P=0.154	14.2 P=0.115	8.30 P=0.504	4.76 P=0.850
AIC	1627	1609	1612	799	785	786	2025	2005	2006
BIC	1695	1680	1684	866	857	858	2092	2077	2078
Likelihood ratio	Reference	P<0.001	P<0.001	Reference	P<0.001	P<0.001	Reference	P<0.001	P<0.001
Reclassification									
Category NRI (95% CI)									
Event	Reference	0.022 (−0.051 to 0.083)	−0.037 (−0.060 to 0.077)	Reference	0.188 (−0.031 to 0.193)	0.145 (−0.030 to 0.238)	Reference	0.030 (−0.053 to 0.067)	0.018 (−0.057 to 0.063)
Nonevent	Reference	0.113 (0.025–0.188)	0.081 (−0.009 to 0.166)	Reference	0.095 (0.016–0.126)	0.085 (0.010–0.119)	Reference	0.094 (0.021–0.206)	0.122 (0.019–0.205)
All	Reference	0.136 (0.004–0.248)	0.044 (−0.009 to 0.220)	Reference	0.284 (0.005–0.296)	0.230 (0.003–0.328)	Reference	0.124 (0.003–0.239)	0.141 (−0.007 to 0.238)
Continuous NRI (95% CI)									
Event	Reference	0.162 (0.064–0.348)	0.250 (0.075–0.369)	Reference	0.159 (0.013–0.406)	0.217 (0.070–0.457)	Reference	0.145 (0.023–0.295)	0.181 (0.065–0.305)
Nonevent	Reference	0.196 (0.100–0.326)	0.150 (0.042–0.269)	Reference	0.179 (0.047–0.346)	0.182 (0.056–0.373)	Reference	0.176 (0.086–0.283)	0.143 (0.045–0.237)
All	Reference	0.358 (0.197–0.645)	0.400 (0.145–0.610)	Reference	0.338 (0.111–0.706)	0.400 (0.168–0.765)	Reference	0.320 (0.137–0.547)	0.324 (0.129–0.512)

Continued

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Table 4. Continued

Performance Measures	All-Cause Mortality			Cardiovascular Mortality				MACE			
	Clinical Model	Clinical Model Plus log_2-NT-IGFBP-4	Clinical Model Plus log_2-CT-IGFBP-4	Clinical Model	Clinical Model Plus log_2-NT-IGFBP-4	Clinical Model Plus log_2-CT-IGFBP-4	Clinical Model	Clinical Model Plus log_2-NT-IGFBP-4	Clinical Model Plus log_2-CT-IGFBP-4
IDI (95% CI)									
Event	Reference	0.025 (0.009–0.055)	0.022 (0.005–0.050)	Reference	0.027 (0.003–0.069)	0.030 (0.007–0.071)	Reference	0.022 (0.007–0.048)	0.020 (0.006–0.044)
Nonevent	Reference	0.007 (0.002–0.015)	0.005 (0.001–0.013)	Reference	0.002 (0.000–0.008)	0.003 (0.001–0.009)	Reference	0.006 (0.002–0.016)	0.007 (0.002–0.015)
All	Reference	0.033 (0.011–0.071)	0.027 (0.006–0.063)	Reference	0.029 (0.004–0.077)	0.033 (0.008–0.079)	Reference	0.028 (0.009–0.063)	0.027 (0.008–0.059)

A clinical model was generated based on variables selected for model 1 (age, sex, estimated glomerular filtration rate, hypertension, high-density lipoprotein, total cholesterol, systolic blood pressure, hemoglobin, current smoking, diabetes, previous acute myocardial infarction, multivessel coronary artery disease, complex lesion, C-reactive protein, and peak troponin I). The baseline model was then extended by the log_2-transformed NT- or CT-IGFBP-4 variable. For category NRI, patients were divided into risk categories (<5%, 5–10%, 10–20%, and >20%) and reclassified. AIC indicates Akaike information criterion; BIC, Bayesian information criterion; CT, C-terminal; H-L, Hosmer–Lemeshow; IDI, integrated discrimination improvement; IGFBP-4, insulin-like growth factor binding protein 4; MACE, major adverse cardiac event; NRI, net reclassification improvement; NT, N-terminal.

challenging, Pencina et al[27] suggested interpretations of the continuous NRI: NRI <0.2 is weak, NRI ≈0.4 is intermediate, and NRI >0.6 can be considered strong. Continuous NRI in the present study ranged between 0.33 and 0.40.

Biomarkers that mirror the degree and severity of the plaque burden are especially interesting because they may also be useful in syndromes such as unstable angina pectoris, in which peak TnI and creatinine kinase–MB levels are not elevated and ECG changes are often lacking or inconclusive. The rationale for the use of IGFBP-4 fragments as biomarkers for cardiac risk assessment is based on the assumption that PAPP-A is actively involved in the development of atherosclerosis.[12,13] It has been suggested, however, that IGF-1 and PAPP-A may instead be cardioprotective and increase as a compensatory mechanism attempting to limit plaque progression and overall atherosclerotic burden.[31,32] Speaking against this theory, apolipoprotein E–deficient mice with a deletion of the PAPP-A gene and fed a high-fat diet show an 80% reduction in plaque area, whereas transgenic overexpression of PAPP-A accelerates plaque progression.[10,11,33] Moreover, PAPP-A substrate binding site inhibition with a neutralizing monoclonal PAPP-A antibody results in 70% reduction in atherosclerotic plaque development in apolipoprotein E–deficient mice.[34] Accordingly, PAPP-A seems to play an unfavorable functional role in the process of plaque destabilization and may not only be a marker of plaque vulnerability but also be indicative of poor prognosis after the occurrence of a cardiovascular event.[1,4] The use of the IGFBP-4 fragments as biomarkers is further supported by a number of observations of which a few merit particular attention. First, we recently showed that circulating levels of PAPP-A correlate with levels of NT- and CT-IGFBP-4 fragments,[12] and this was confirmed in the present study. Second, PAPP-A–dependent proteolysis of IGFBP-4 is very specific, and the IGFBP-4 fragments do not undergo further modifications or truncations in the circulation.[35] Third, the IGFBP-4 fragments display great storage stability.[5,13] Fourth, treatment of patients with heparin promptly increases circulating PAPP-A levels,[4,12] whereas the 2 IGFBP-4 fragments are not affected. Thus, heparin may displace cell-surface associated PAPP-A, but the overall PAPP-A activity toward IGFBP-4 appears unaltered.[5] To reaffirm this finding in the present study, PAPP-A levels were measured in 38 randomly selected patients, and levels were significantly higher in all patients (>20 ng/mL) compared with non–heparin-treated participants (<2 ng/mL).[12] Finally, active PAPP-A consists of 2 identical PAPP-A subunits, whereas the inactive form is composed of 2 PAPP-A subunits covalently linked to 2 inhibitory subunits of the proform of eosinophil major basic protein.[7,36] PAPP-A assays, however, generally lack the ability to discriminate between the forms.[37,38]

So far, studies of IGFBP-4 fragments as cardiac risk markers in patients have provided conflicting results. In a study by Schulz et al,[39] NT- and CT-IGFBP-4 failed to predict long-term outcome in patients with stable CVD, whereas Postnikov et al[13] showed strong associations between the fragments and short-term cardiac events in patients with acute myocardial infarction. It has been proposed that the prognostic value might depend on whether patients present with acute or stable disease, and it is intuitive to speculate that IGFBP-4 fragment levels merely recapitulate infarct size and the acute situation. However, we recently demonstrated that NT- and CT-IGFBP-4 levels were not altered during the acute phase of a myocardial infarction,[5] and the fragments predicted cardiovascular mortality in type 1 diabetes patients without CVD at baseline during 12 years of follow-up.[12] These observations suggest that the discrepancies cannot solely be explained by acute versus stable disease or short versus long follow-up. We speculate that the different outcomes relate to differences in study populations. It is worth mentioning that in neither this nor previous studies did we find an association between the NT- or CT-IGFBP-4 fragments and LVEF, peak TnI, location and complexity of the culprit lesion, symptom-to-balloon time, or the use of statins and antiplatelet agents, such as glycoprotein IIb/IIIa and cyclooxygenase inhibitors.[5,12]

Although our findings are interesting and potentially clinically relevant, the specific mechanisms in STEMI patients are elusive and warrant further investigation. Furthermore, we acknowledge that the value and utility of biomarkers in STEMI patients is limited compared with other CVDs. One could argue that the IGFBP-4 fragments may be more useful for risk stratification of patients with unstable angina or non-STEMI. Instead, the potential purpose of a biomarker in STEMI may be to provide long-term prognostication in patients at hospital discharge in the hope of optimizing treatment management. Considering this perspective, the present findings suggest that the prognostic value of the IGFBP-4 fragments may be complementary to other risk factors and improve clinical decision making. However, our results cannot necessarily be extrapolated to other patient cohorts, and clinical adoption should await further validation of the ability of the biomarkers to advise therapy that improves patient outcomes.

Study Strengths and Limitations

The cohort size and the follow-up period resulted in a large number of events, which allowed for extensive adjustment. As for study limitations, samples were collected only at baseline and not throughout the follow-up period; therefore, we were unable to evaluate changes in variables over time. The study was entirely observational and yielded no evidence of causality. Unfortunately, LVEF measurements were not obtained for all patients and would have strengthened the analyses. Finally, comparisons with additional relevant biomarkers, such as B-type natriuretic peptide, were not incorporated into the design and are beyond the scope of this study.

Conclusions

Elevated levels of PAPP-A–generated IGFBP-4 fragments are significantly associated with increased risk of all-cause mortality, cardiovascular mortality, and MACE in patients with STEMI. Especially with regard to cardiovascular mortality, the IGFBP-4 fragments possess incremental prognostic value beyond that of conventional clinical risk factors.

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Disclosures

None.

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Supplemental Material

Table S1. Reclassification table comparing 5-year risk of cardiovascular mortality before and after addition of NT-IGFBP-4 to the clinical model.

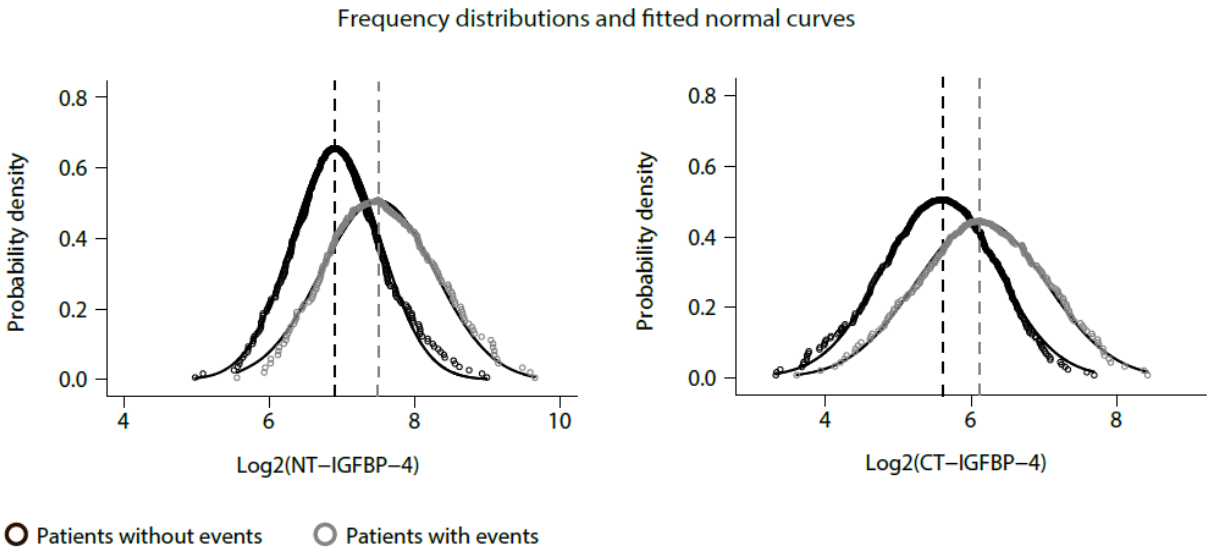
Patients were divided into risk categories (<5%, 5-10%, 10-20% and >20%) and classified using a clinical model (age, sex, eGFR, hypertension, HDL, total cholesterol, systolic blood pressure, haemoglobin, current smoking, diabetes, previous AMI, multivessel coronary artery disease, complex lesion, CRP and peak TnI) with and without the addition of NT-IGFBP-4. Total NRI was the sum of NRI in patients with and without events, using the calculation: ((number of events classified upwards) + (number of events classified downwards))/total number of events + ((number of non-events classified downwards) + (number of non-events classified upwards))/total number of non-events. Light gray: improved classification, white: no classification change, dark gray: worse classification.

AMI, acute myocardial infarction; CRP, C-reactive protein; CT, C-terminal; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; NRI, net reclassification improvement; NT-IGFBP, N-terminal insulin-like growth factor binding protein; TnI, Troponin I.

Risk classification using clinical model	Risk classification using clinical model + log_2-NT-IGFBP-4					Reclassified	NRI
	<5%	5-10%	10-20%	>20%	Total		
Patients with events (n)							
<5%	7	5	0	0	12	+5	13/69 = 0.188
5-10%	1	5	5	0	11	+4	
10-20%	0	1	5	8	14	+7	
>20%	0	1	2	29	32	-3	
Total	8	12	12	37	69	+13	
Patients without events (n)							
<5%	285	19	1	0	305	-20	56/587 = 0.095
5-10%	65	50	18	4	137	+43	
10-20%	5	24	44	11	84	+18	
>20%	0	2	13	46	46	+15	
Total	355	95	76	61	587	+56	
Total NRI							0.284

Figure S1. Frequency distribution of log_2-NT- and log_2-CT-IGFBP-4 and fitted normal curves.

Frequency distributions of log_2-NT- and log_2-CT-IGFBP-4 in the 431 patients without events (black circles) and the 255 patients with events (gray circles), along with the fitted normal curves (black). The vertical lines mark the mean protein levels. CT, C-terminal; IGFBP, insulin-like growth factor binding protein; NT, N-terminal.



Insulin–Like Growth Factor Binding Protein 4 Fragments Provide Incremental Prognostic Information on Cardiovascular Events in Patients With ST–Segment Elevation Myocardial Infarction

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